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HUMAN PARVOVIRUS B19 AND RHEUMATOID ARTHRITIS

Takeshi Sasaki

Abstract Human parvovirus B19 (B19) is single stranded DNA virus, that causes erythema infectinosum in infant and/or acute onset polyarthritis in adult. We present the evidence showing the role of B19 on the etiopathogy of rheumatoid arthritis (RA). (1) B19 DNA could be frequently amplified in the samples from rheumatoid joints. The detection B19 RNA and B19 protein VP1 was specific for RA, and positive at T cells, B cells, macrophages and follicular dendritic cells in rheumatoid synovium. (2) B19 infection or transduction of B19 NS1gene caused TNF α, IL-6 and IL-8 production through activating AP1 and AP2 in macrophages or macrophage celll line U937. We also found Ku80 as a novel receptor for B19 on T cells, macrophages or erythroblasts. B19 used clathrin on the surface at their cell entry and caused enhanced actin polymerization, resulting in the migration of T cells. (3) B19-transgenic mice became susceptible to type II collagen-induced polyarthritis that is a model of RA. We also experienced 12 cases who developed RA after acute B19 infection. (4) Half of RA cases had a defective neutralizing ability to B19.

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Key words: human parvovirus B19; rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic polyarthritis with destructive joints, where a variety of cytokines has an important role in the inflammatory process. The etiology of RA is unknown. In the past decade, extensive studies have focused on defining the genes associated with the occurrence of RA; the studies included genetic polymorphisms of immunologically related genes, such as MHC. Microenvironment is another factor that may be responsible for the etiopathogenesis of RA, and human parvovirus B19 (B19) is one candidate in the etiopathogenesis as indicated by epidemiological data showing that B19 infection and RA may be relatively new diseases in Europe[1-3]. B19, that is a causative agent of erythema infectiosum in infants[4], often causes acute onset polyarthritis in adults[5,6] that, in some cases, may resemble RA in terms of human leukocyte associated antigen, rheumatoid factor production and clinical signs including erosive change of joints[7-10]. This paper presents the role of B19 in the pathogenesis of RA.

1. Clinical feature of human parvovirus B19 infection

Human parvovirus B19 belongs to the family *Parvoviridae* and the genus *Erythrovirus*. The B19 genome includes three major open reading frames coding for the nonstructural protein NS1 in the left half and structural proteins VP1 and VP2 in the right half. B19 is the only parvovirus that has been clearly linked with disease in humans. Common manifestations caused by B19 infection include transient aplastic crisis in patients with histories of chronic hemolytic anemia, erythema infectiosum[4], nonimmune hydrops fetalis[12], chronic pure red cell aplasia in patients with immunosuppression. The onset of polyarthritis is common at B19 infection in adults (mostly woman)[5,6]. Joint symptoms last for 1 to 3 weeks, although they may persist for months or years. B19 arthritis often meets clinical diagnostic criteria for RA and can be erosive[8-13].

Department of Rheumatology and Hematology,
Tohoku University Graduate School of Medicine,
1-1 Seiryocho 1-1, Aobaku , Sendai, 980-8574, Japan

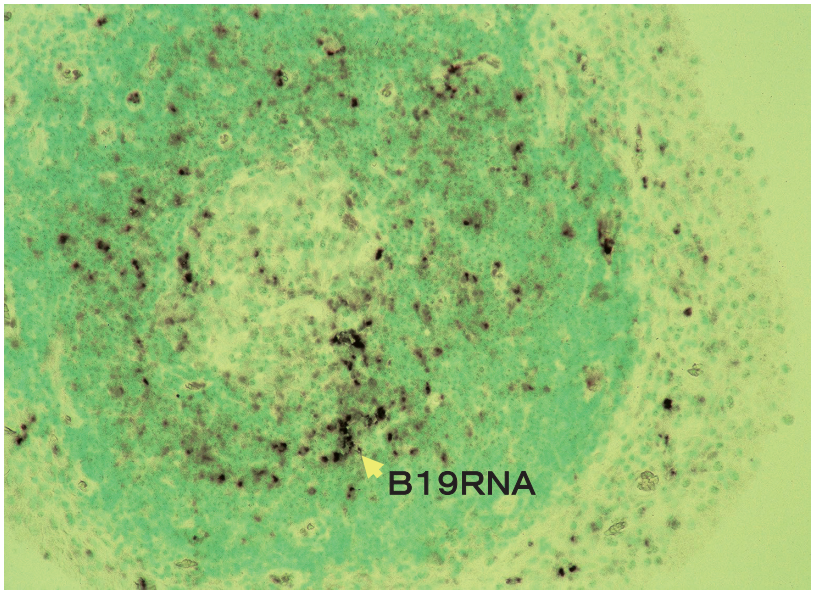

Figure 1 B19 in RA synovium cells. The specimen from RA synovium was stained for B19 RNA through in situ hybridization. Dark granules show B19 RNA-positive.

2. B19 in rheumatoid joints

(1) Detection of B19 DNA/RNA and B19 protein in the cells of rheumatoid joints.

The B19 RNA and B19 structure protein, VP1, are specifically and frequently detected in T and B cells, macrophages and follicular dendritic cells of the rheumatoid synovium, when determined by PCR, in situ hybridization and histoimmunochemical studies[14]. Coculture study of rheumatoid synovial cells with uninfected macrophage cell line U937 or bone marrow cells using a double chamber system showed that tumor necrosis factor alpha (TNFα) and IL-6 were secreted from the cocultured cells, and the production of the cytokines was inhibited by anti-B19 antibody recognizing viral protein 1 (VP1) [14].

(2) Isolation of the B19 genome from the rheumatoid synovium

We amplified the almost whole genome of B19 isolated from the synovial tissues of two patients (Mi and Rm) with RA. The amplified sequences of B19 DNA from rheumatoid synovium of Mi were completely coincided with those from bone marrow of the same individual[15]. A higher similarity was found between RA-derived isolate Mi and aplastic crisis isolate N8 with only two amino acid differences. Further analysis using DNA samples from the four more patients with RA and from 16 patients with acute B19 infection, however, revealed that no genetic change was specific among RA-derived isolates (data not shown).

3. B19 causes the activation of T cells and macrophages.

(1) Receptor for B19 infection on immunocytes

B19 were positive on T, B cells or macrophages in rheumatoid synovium in spite of poor or no expression of P antigen that has been believed only receptor for B19[16]. We speculated the putative unknown receptor for B19 on T cells or macrophages. Immunofluorescence study using FITC-labeled B19 empty particles revealed that B19 bound not only human erythroid cells with surface P antigen, but also non-erythroid cells which may lack P antigen on their surface.

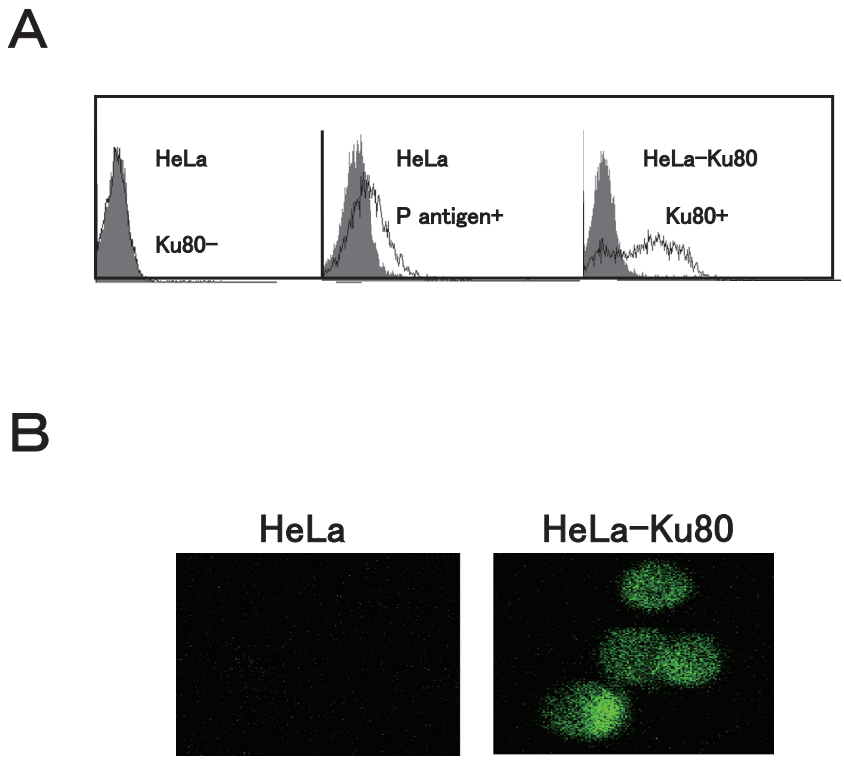

Figure 2 Ku 80 as a cellular receptor for B19. A: HeLa cellsthat were tranduced with/without Ku80 gene were stained for surface expression of Ku80. B: B19 was infected to Ku80-transduced or nontransduced HeLa cells in vitro, and then stained for the presence of B19 protein.

The molecule responsible for B19 binding at noneryhtroid cells was Ku80 antigen. Western blotting analysis, competitive ELISA, and flow cytometry studies showed specific binding of B19 to Ku80. Furthermore, transfection of HeLa cells with the Ku80 gene enabled the binding of B19 and allowed its entry into cells[17]. Although Ku80 originally has been described as a nuclear protein, human bone marrow erythroid cells with Glycophorin A or CD36, B cells with CD20 or T cells with CD3 were all positive for cell surface expression of Ku80. B19 infection of erythroblastoid cell line and bone marrow cells was inhibited in the presence of anti-Ku80 antibody. This suggests that Ku80 functions as a novel coreceptor for B19 infection, and this finding provides an explanation for the pathological immunity associated with B19 infection.

(2) B19 infection enhances of immunocytes

B19 are positive in activated T cells in circulation, and the polymerization of actin was observed in B19-infected T cells. Similarly, actin polymerization occurred in Jurkat cells as well as in H9 T cell line[18]. We could also find similar polymerization by the stimulation with anti-Ku80 antibody. However, anti-Ku70 stimulation did not cause the polymerization. B19 infection also enhances the expression of adhesion molecules such as CD29 and CD11a in T cells. Enhanced actin polymerization and adhesion molecules may leads the enhanced migration activity in T cells. The resulting migration assay using transwell system revealed an enhanced migration of T cells[18]. Early event of RA is the invasion of T cell or macrophages from circulation to synovium. Why rheumatoid T cells or macrophages infiltrate to the tissue has been unknown. These data indicate that B19 may have a role for the infiltration through activation of migration.

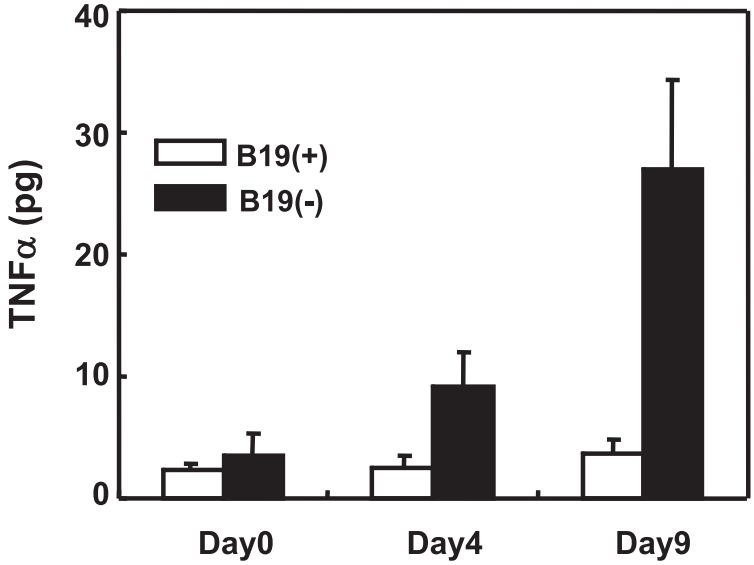

Figure 3 An enhanced production of TNF α by U937 through B19 infection.

(3) B19 causes the production of inflammatory cytokines

It is important to know whether the transmission of B19 from rheumatoid synoviocytes to monocytic cells induce an enhanced secretion of TNFα, which is assessed to trigger the inflammation at RA joints. To determine the role of B19 in the production of TNFα, we had two experimental system. One is to know the ability of macrophages to produce TNFα at B19 infection. This was proved at the in vitro infection of B19 to macrophage cell line U937 where B19-infected U939 caused the production of TNFα[19].

Next, we focused on the function of its nonstructural protein, NS1, and established monocytic U937 lines transduced with the NS1 gene under the control of an inducible promoter. Production of TNFα mRNA and protein was elevated in a manner associated with NS1 expression. Reporter assays revealed that AP-1 and AP-2 motifs on the TNFα promoter were responsible for NS1-mediated up-regulation. Electrophoretic mobility shift assay showed specific binding of nuclear proteins from NS1 gene-transduced cells with the AP-1 or AP-2 probe. Antibodies against transcription factors AP-1 and AP-2 and anti-NS1 antibody inhibited the binding of nuclear proteins to the corresponding probes. These data indicate that NS1 up-regulates TNFα transcription via activation of AP-1 and AP-2 in monocytic cells[20]. The molecular mechanisms of NS1-mediated TNFα expression may explain the pathogenesis of B19-associated inflammation.

4. Induction of rheumatoid arthritis by B19

(1) Induction of polyarthritis resemble RA in B19 transgenic mice.

To understand the role of B19 in polyarthritis, we generated transgenic mice expressing the B19 gene from rheumatoid joints, and investigated the ability of B19 to induce polyarthritis associated with inflammatory cytokines. The NS1 gene encodes a functional protein of B19, and, therefore, we used the nonstructual protein 1 (NS1) gene of the Mi isolate derived from RA, to construct the transgene for B19 transgenic mice, and NS1gene into C57BL/6 mice that had a genetic origin not susceptible to arthritis. The transgenic mice developed no lesions spontaneously, but

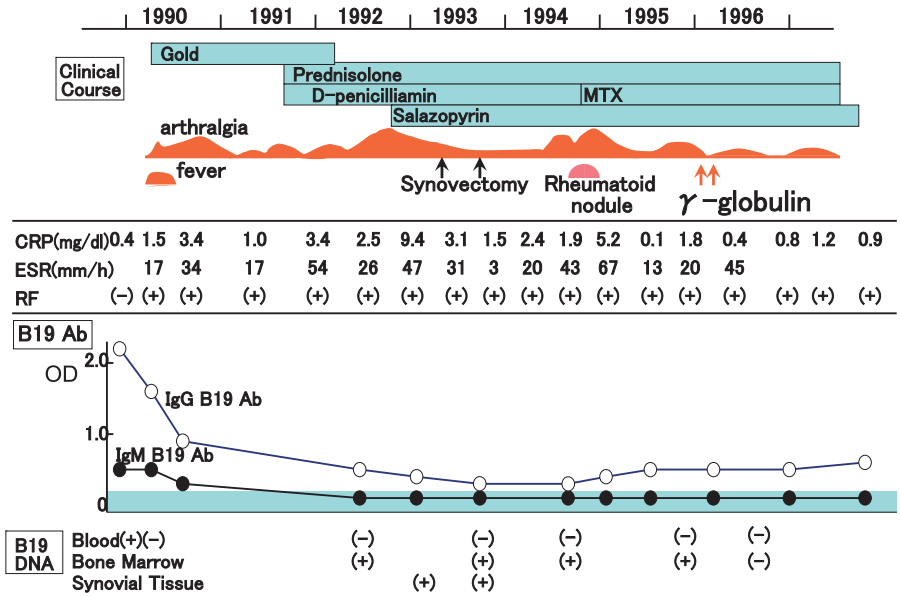

Figure 4 A case who developed RA after acute B19 infection.

were susceptible to type II collagen (CII) -induced arthritis. B19 NS1 was expressed in synovial cells on the articular lesions that were histologically characteristic of granulomatous synovitis and punnus formation in cartilage and bone[15]. Serum levels of anti-CII antibodies and TNFα increased in NS1 transgenic mice to the same levels as those of DBA/1 mice, which were susceptible to polyarthritis. Stimulation with CII increased secretion of Th1 type and Th2 type cytokines in NS1 transgenic mice, indicating that a nonpermissive H-2^b haplotype in wild type of C57BL/6 mice can be made susceptible to polyarthritis through the expression of NS1. This study is the first to show that a viral agent from the joints in humans can cause CII-induced arthritis resembling RA.

(2) Development of rheumatoid arthritis after acute B19 infection in humans.

Eleven patients developed RA after acute B19 infection, and we therefore compared clinical features of them with 20 RA patients who had not the evidences of human parvovirus B19 (B19) infection at the onset of RA[13,21,22]. The result revealed no differences between the clinical profile of patients with B19 and that without B19, indicating that B19 may cause RA at least in some population with RA.

5. Possible mechanism for B19-associated rheumatoid arthritis

The above mentioned results indicates the role of B19 in the etiopathology of RA. In most patients with acute B19 infection, B19 disappears soon after clinical appearance of polyarthritis, that is also transient[5,6]. On the other hand, B19 protein VP1 and B19 DNA and RNA were detectable repeatedly at different times in macrophages, follicular dendritic cells and T and B cells in the rheumatoid synovium, indicating persistent activation of B19 in the rheumatoid synovium. The secretion of TNFα and IL-6 induced by the coculture of macrophage cell line U937 or THP1 with rheumatoid synovial cells was inhibited by coexistence of monoclonal anti-B19 antibodies[14]. We also showed that transduction of U937 cells with NS-1 gene activated transcription factors AP-1 and AP-2, resulted in the upregulation of TNFα gene expression and secretion of TNFα from host cells[20]. This

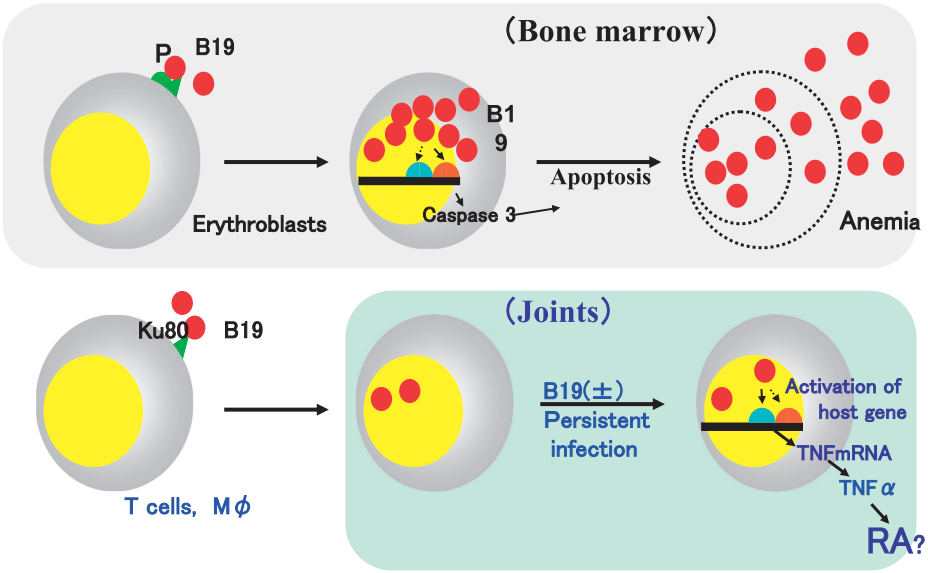

Figure 5 B19 in cells. B19 infects and proliferates in erythroid cells. The markedly increased B19 may be killed by anti-B19 antibodies. On the other hand, B19 proliferate very slowly in T cells or macrophages, but escape from the attack by immune cells because of intracellar infection. NS1 protein derived form persistently infected B19 will activate host genes responsible for inflammatory cytokines.

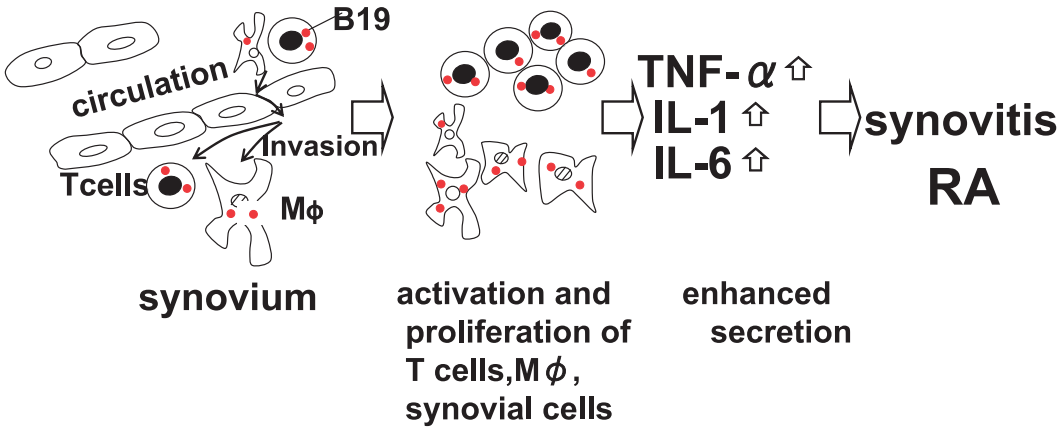

Figure 6 Possible pathogenesis of RA. B19 infected T cells or macrophages may invade into synovium, proliferate, and produce a variety of inflammatory cytokines, resulting in synovitis in joints.

indicates that persistent activation of B19 may constantly upregulate the TNFα gene in infected cells. Data of the NS1-transgenic model leads us to hypothesize that persistently activated B19 may induce an increased immune response, such as activating and proliferating synoviocytes by an autocrine and paracrine pathway in the joints, resulting in an inflammatory process.

This may explain the inflammatory mechanism of RA. The clinical course of RA is long and different immune cells continuously proliferate and secrete marked amounts of cytokines in RA joints where TNFα would have a central role in the inflammatory process of the disease. Our results indicate that B19 may be responsible for the etiopathogenesis of RA at least in some population.

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